(*NEW SERIES.*)

No. 16.

SCIENTIFIC MEMOIRS

BY

OFFICERS OF THE MEDICAL AND SANITARY DEPARTMENTS

OF THE

GOVERNMENT OF INDIA.

THE SPECIFICITY OF ANTIVENOMOUS SERA WITH SPECIAL REFERENCE TO A SERUM PREPARED WITH THE VENOM OF DABOIA RUSSELLI.

BY

CAPTAIN GEORGE LAMB, M.D., I.M.S.

ISSUED UNDER THE AUTHORITY OF THE GOVERNMENT OF INDIA
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The Specificity of antivenomous Sera, with special reference to a serum prepared with the venom of Daboia Russellii.

In two previous communications[1] on the specificity of antivenomous sera I brought forward evidence which pointed to the conclusion that a pure antivenomous serum, that is, a serum prepared with a single poison, is markedly, if not absolutely, specific for the venom which has been used in its preparation. This conclusion was based on the results of the testing of two antivenomous sera, one prepared with pure cobra poison and the other with the pure poison of the Australian tiger snake (Hoplocephalus curtus), against the general actions *in vivo*, the hæmolytic actions *in vitro* and the actions on the coagulability of the blood plasma also *in vitro*, of ten different venoms, some of which were the poisons of colubrine species while others were of viperine origin. It is unnecessary to enter into the details of the experiments which were then carried out and which have been set forth in the papers above mentioned. Before, however, passing on to the subject matter proper of the present communication, to wit, some observations made with the object of the testing of a serum prepared with a pure viperine venom, that of Daboia Russellii, I have to refer briefly to the results which were obtained *in vivo* with the cobra venom anti-serum and the poisons of some of the other colubrine snakes against which it was tested. This reference is necessary in view of the fact, that the results which have been obtained by Captain Rogers[2] with Calmette's serum differ from those which I have obtained with the serum prepared with pure cobra venom. It is, however, to be remembered in this connection that Calmette's serum is made by vaccinating with a mixture of venoms, of which mixture cobra venom is the preponderating constituent. This fact alone might have been brought forward as an adequate explanation of the differences between my results and those obtained by Captain Rogers, had not this observer in a more recent paper [3] stated that, using a serum prepared in London by Messrs. Burroughs and Wellcome with pure cobra venom, he obtained similar results to those which he had previously got with Calmette's serum. Further, in a letter to the 'Lancet' Captain Rogers [4] has recently stated, that Dr. Calmette has told him that the serum with which he was supplied and which he used for his experiments happened to be prepared with pure cobra venom. His experiments, therefore, are strictly comparable with those which I have made with a pure cobra venom anti-serum. In what then do the differences consist, and how are they to be explained?

In the first place, let us consider the experiments with the venom of the king cobra, a snake belonging to the same genus as the cobra. Captain Rogers, working with a serum, 1 c.c. of which, he showed, was able to neutralise 0·56 milligramme of cobra venom, found that the same quantity of serum could completely neutralise 0·3475 milligramme of the venom of the king cobra, the toxicity of which poison is practically the same as that of cobra venom. Now, in my experiments with a cobra venom anti-serum, 1 c.c. of which was able to neutralise 1·5 milligrammes of its homologous poison, it was found that, while large amounts of serum were able to delay death in cases of intoxication with the venom of the king cobra, no complete neutralisation took place: that 1 c.c. of serum could not neutralise even 0·13 milligramme of this poison. It will be seen, therefore, that while the serum I used was, as far as cobra venom was concerned, nearly three times as strong as that employed by Captain Rogers, it failed to neutralise about a third of the amount of king cobra venom which this observer found Calmette's serum could effectually neutralise.

It is to be noted that in both series of experiments the sera were tested against ten lethal doses of venom, but that while Captain Rogers used pigeons I employed rabbits. I have recently repeated the observations which bear on this point. In these experiments a serum prepared with pure unheated cobra venom was again employed: 1 c.c. of this serum was able to neutralise 0·7 milligramme of pure cobra venom. It was now tested against only 6 lethal doses of king cobra venom by subcutaneous injection into rabbits, the serum and venom being allowed to stand *in vitro* at room temperature for half an hour before injection. A reference to the protocols (table I) will show that even 15 c.c. of serum could not save the life of the animal. We can, moreover, calculate that 1 c.c. of serum had failed to neutralise 0·11 milligramme of this poison. From this series of experiments, therefore, we arrive at the same conclusion as we had come to previously, namely, that, while a serum prepared with pure cobra venom and strongly antitoxic for this venom delays death in cases of intoxication with the venom of the king cobra, it does not completely neutralise this poison even when used in large quantities.

It is difficult to reconcile my results with those obtained by Captain Rogers. It is, however, to be noted that Captain Rogers' experiments were made with pigeons while mine were carried out with rabbits. It might be conceivable that a certain action of the poison in mammals was wanting in birds, and that the constituent which caused this action remained unneutralised in my experiments. That all the constituents of king cobra venom are not neutralised by a cobra antivenom is evident, as I have shown,[5] from the fact that such a serum has no hindering effect on the hæmolytic action of king cobra poison when tested *in vitro*. Further, Rogers does not definitely state that in his experiments the

minimum lethal dose of the venom was determined for pigeons, the animals on which the serum experiments were carried out: in fact, by stating that, on account of a difficulty in obtaining rats, pigeons were used for the serum experiments, he leads one to suppose that the minimum lethal dose had been determined for rats and not for pigeons. It is possible, therefore, that in his serum experiments with pigeons he was using not ten lethal doses but a very much smaller number. The only other possible explanation that I can think of is that the poison with which Captain Rogers was working was not that of the king cobra. I may state that the king cobra venom which I used was collected directly under my own supervision or that of Colonel Bannerman, I.M.S., from snakes the property of the Natural History Society, Bombay.

In the second place we have to consider the experiments with the venom of Bungarus cœruleus or common krait.

Working with Calmette's serum Captain Rogers found that 1 c.c. could neutralise 0·072 milligramme of krait poison. In my experiments it was found that 1 c.c. of pure cobra venom anti-serum, which was nearly three times the strength of the serum used by Captain Rogers, failed to neutralise 0·0925 milligramme of the venom of Bungarus cœruleus and that, moreover, the serum had no effect on the progress of the intoxication.

I have recently repeated these experiments, again using a pure cobra anti-venom, 1 c.c. of which was able to neutralise 0·7 milligramme of cobra poison. Employing in these experiments as a test dose only 5 lethal doses by intra-venous injection into rabbits, I found that 8 c.c. of serum had no effect whatever in even prolonging life (protocols, table II). We can, therefore, calculate that 1 c.c. of this serum failed to neutralise 0·025 milligramme of venom, a result strictly comparable to that previously arrived at.

A careful perusal of Captain Rogers' paper shows that a serious objection can be at once raised to his experiments. For, it is seen that, while he determined the minimum lethal dose for pigeons, he used rats in the serum experiments, evidently presuming the venom to be of the same toxicity for these latter animals as for pigeons. Thus, he found the minimum lethal dose for pigeons to be 0·25 milligramme per kilo., and accordingly in the serum experiments he used as a test dose 2·5 milligrammes per kilo. for rats. Now, in a more recent paper [6] from Professor Fraser's laboratory, Elliot, Sillar and Carmichael have accurately determined the minimum lethal dose of this venom for rats and have found it to be one milligramme per kilo. It is, therefore, apparent that in his serum experiments Captain Rogers was using as a test dose only $2\frac{1}{2}$ lethal doses instead of ten lethal doses as he supposed. Further, it is doubtful whether he was working with pure krait venom. For, as a result of his experiments on the physiological actions he states, that the symptoms following poisoning by this venom are identical with

those following poisoning by cobra venom, whereas the results obtained by the three workers mentioned above indicate, "that while the symptoms are similar, still they differ so much in relative degree as to render it doubtful if they can in future be spoken of as identical." Further, I hope to show at a later date that the action of this venom on the central nervous system is quite different from that of the poison of the cobra. I may state that the krait venom which I used for my observations was collected at the Plague Research Laboratory, Bombay, either under my own supervision or that of Colonel Bannerman.

As regards the experiments with the venoms of the other colubrine snakes Captain Rogers and I are in agreement, namely, that in relatively large amount a cobra venom anti-serum is able to neutralise *in vivo* the poison of one of the sea snakes, Enhydrina vaiakadien, while it delays death but does not neutralise the venom of Bungarus fasciatus. In this latter case Captain Rogers is of opinion that the colubrine constituents of the venom are neutralised, the animal dying of chronic viperine poisoning. To this point I shall again refer in a paper, to be written along with Dr. Hunter, on the action of viperine poisons on the central nervous system.

From these considerations it seems to me that the conclusions arrived at in my last paper on the specificity of antivenomous sera were quite justified, namely, that a serum of a horse immunised with pure cobra poison is strongly antitoxic for the venom used in its preparation: when used in large quantity it has a slight neutralising power for the venom of Enhydrina valakadien, one of the common sea snakes: further, it delays death in cases of intoxication with the venom of the king cobra, a species belonging to the same genus as the cobra, and also in cases of intoxication with the venom of Bungarus fasciatus: it does not, however, completely neutralise these poisons even when used in large quantities. The serum would be of little or no therapeutic value in cases of bites from these three snakes. Finally, the serum contains no antitoxic substances, which are active against the venom of Bungarus cœruleus. It is to be remembered that these conclusions refer only to the results of the testing of the serum '*in vivo*'. We are not concerned at present with the hæmolytic actions or the actions on the blood plasma of the several venoms as tested *in vitro*.

We may now pass on to the consideration of the subject matter proper of the present communication, that is, some observations which were made with an anti-serum for the venom of Daboia Russellii. This serum was prepared by injecting a horse with gradually increasing doses of the pure unheated poison of Russell's viper. The treatment lasted over a period of one year. From a dose of one milligramme the amount given at each injection was gradually increased until at the time of the testing of the serum

a dose of 500 milligrammes could be given without causing any disturbance other than considerable local reaction. The serum was now tested against the same ten poisons as were used in the testing of the cobra venom anti-serum. Further, the same plan of testing was adopted in this case as was employed on the previous occasion. Thus, in the first place, the serum was tested against the general actions *in vivo* of the several poisons: in the second place, it was tested against their hæmolytic actions *in vitro* and, in the third place, it was tested against the actions of several of the poisons on the coagulability of the blood plasma also *in vitro*. It will be seen, therefore, that in this case also the serum has been tested in a fairly exhaustive manner.

Let us first consider the experiments made with the object of ascertaining if the serum had any neutralising effect on the general actions *in vivo* of the various poisons.

Colubrine snakes.—The serum was tested against the venoms of five colubrine snakes, namely, cobra, king cobra, Bungarus cœruleus, Bungarus fasciatus and Enhydrina valakadien (Sea-snake). The results of these observations are detailed in the protocols (table III). From these it will be seen that, as was to be expected, although comparatively large amounts of serum were used, no neutralising action was exhibited by the serum in any instance.

Viperine snakes.—The poisons of four viperine species were available for testing the serum. These were, (1) Daboia Russellii, (2) Echis carinata (*phoorsa*), (3) Trimeresurus gramineus (Green pit viper) and (4) Crotalus adamanteus (the American rattlesnake).

Daboia Russellii.—A series of careful experiments was made with this poison in order to standardise accurately the serum against the venom with which it was prepared. The test dose used was fifty lethal doses by intravenous injection into rabbits, namely, 5 milligrammes per kilo. A reference to the protocols (table IV) will show, that 1·75 c.c. of serum were able to save the life of the animal when mixed with this test dose. Thus, it can be calculated that 1 c.c. of serum would be able to neutralise 2·8 milligrammes of venom. Further, it will also be seen that, while the animal which received 0·75 c.c. of serum along with the test dose of venom died in two minutes, the result of intravascular clotting, the rabbit which got 1 c.c. of serum lived for 10½ hours. In this latter case the serum had evidently neutralised the fibrin ferment of the poison, but had failed to neutralise the constituents which cause the more or less chronic effects. In a previous communication[7] I have stated that the strongest cobra venom anti-serum which I have been able to prepare was one of which 1 c.c. could neutralise 1·5 milligrammes of pure cobra venom, but that as a rule it was only possible to obtain a serum of much less potency than this. It would, therefore, appear that daboia venom yields a stronger serum than cobra venom. The horse from which the present serum was obtained

had been under treatment for only a year, and the maximum amount of venom it had received at one injection was 500 milligrammes.

Echis carinata.—The physiological action of this venom, as far as I have ascertained from a number of experiments, is similar to that of daboia venom. It is, however, of somewhat greater toxicity. It was, therefore, of the greatest interest to ascertain if daboia venom anti-serum had any neutralising effect on the general action *in vivo* of this poison.

In a preliminary series of experiments a test dose of ten lethal doses, namely, 0·5 milligramme per kilo. by intravenous injection into rabbits, was used. As, in these experiments, the serum appeared to have no effect whatever on the action of the poison, a second observation was made in which the test dose used was reduced to two lethal doses. A reference to the protocols, table V, will show, that an animal which received 5 c.c. of serum along with this test dose died in the same time as the control. We can, therefore, calculate that 1 c.c. of serum could not neutralise the general action *in vivo* of 0·012 milligramme of this poison, a quantity more than two hundred times less than the amount of daboia venom which 1 c.c. of the same serum could neutralise. Further, an experiment was made in which a single lethal dose of venom was used: 5 c.c. of serum in this case had no effect in preventing or even delaying death, which took place 5 minutes after the injection. It is evident, therefore, that this serum has no effect on the general action *in vivo* of the venom of Echis carinata. This conclusion is of considerable interest in view of the fact, to be referred to immediately, that the serum has a strong neutralising effect on the hæmolytic action of this poison when tested *in vitro.*

Trimeresurus gramineus.—This snake is a small Indian viper of the family Crotalinæ, the same family to which the American rattlesnake belongs. Unfortunately I have not been able to obtain sufficient poison to enable me to work out its physiological action in any detail. From a few experiments which have been made it can be said that the action of the poison is similar, if not identical, to that of the venom of Crotalus adamanteus: it is not, however, nearly so toxic as this latter poison.

The test dose used to ascertain if daboia venom anti-serum has any neutralising effect on the general action *in vivo* of this poison was 2 milligrammes per kilo. by intravenous injection into rabbits, an amount less than two lethal doses. It was found that 5 c.c. of serum were unable to prevent death in such an experiment (protocols, table VI). We may conclude, therefore, that the serum has no neutralising effect on the action *in vivo* of this poison.

Crotalus adamanteus.—The minimum lethal dose of this venom for a rabbit by intravenous injection was found to be 0·25 milligramme per kilo. The test dose now used to test the serum under experiment was 8 lethal doses, that is to say, 2 milligrammes per kilo. by intravenous injection. It was found that 4 c.c. of serum

prevented death, while 3 c.c. delayed, but did not prevent, a fatal issue (protocols, table VII). We can, therefore, calculate that 1 c.c. of serum would be able to neutralise the general action *in vivo* of at least 0·45 milligramme of the venom of Crotalus adamanteus.

These experiments conclude the observations which have been made with this serum and the various venoms, as far as their general actions *in vivo* are concerned. We have found, that a serum prepared with the pure venom of Daboia Russellii, a typical viperine poison, has no action whatever on any of the colubrine poisons, five in number, against which it was tested : that it neutralises well its homologous venom : that it has a certain, but not very marked, neutralising effect on the venom of another viper, namely, the American rattlesnake : and that it has no antitoxic action for the venom of a closely allied viper, Echis carinata, nor for that of another Indian viper, Trimeresurus gramineus.

We have now to pass on to the consideration of some observations which were made with the object of determining if a daboia venom anti-serum has any hindering effect on the hæmolytic actions *in vitro* of a number of poisons, ten in all. The technique employed was identical with that used in a similar series of experiments, detailed in the last communication, with the anti-serum for cobra venom and that for the poison of Hoplocephalus curtus. The following is a short summary of the method. The red cells of the dog, as being very susceptible to hæmolysis by all venoms, were chosen. The blood was first gently defibrinated : the red cells were then carefully washed several times with salt solution (0·85 per cent.), being centrifugalised between each washing. A 5 per cent. suspension of the washed red cells was then made in sterile 0·85 per cent. salt solution. It was found that these manipulations completely removed all serum complement. As a preliminary measure it was necessary to determine the minimum complete hæmolysing dose of each venom for 1 c.c. of the 5 per cent. red cell suspension, when a fixed amount of serum complement was added. The complement used was that of the dog, and the amount employed was 0·5 c.c. of a two-fold dilution of fresh serum. The tubes were placed in the incubator (37° C.) for one hour and then in the ice chest over night. Working in this way the hæmolysing value of each poison was accurately determined, and it was easy to fix the minimum amount of venom which could bring about complete hæmolysis of a fixed quantity of red cells. This amount, or a very small multiple of this amount, was the quantity of venom which was used as a test dose to determine whether or not daboia venom anti-serum had any hindering effect on the hæmolytic actions of the various poisons. The test dose of venom dissolved in 0·85 per cent. salt solution was mixed with varying amounts of the serum and the mixtures were allowed to stand at laboratory temperature for at least half an hour. To each tube was then added 1 c.c. of a 5 per cent. suspension of dog's washed red cells and 0·5 c.c. of

a two-fold dilution of dog's fresh serum. The preparations were then treated as described above.

A reference to the protocols (table VIII) will show at a glance the results obtained. In the first place, it was found that this serum had no hindering effect whatever on the venom of any of the six colubrine snakes against which it was tested. In the second place, it was found that it neutralised equally well the venom with which it was prepared and the venom of Echis carinata: that it had a considerable but less neutralising effect on the venom of the American rattlesnake, but no hindering effect on the venom of Trimeresurus gramineus.

These results are of considerable interest and call for some comment. We have previously seen that this serum has a certain neutralising effect on the general action *in vivo* of the venom of Crotalus adamanteus. We now find a corresponding effect on the hæmolytic action *in vitro* of this poison. We have also seen that it has no neutralising effect on the general action *in vivo* of the venom of Echis carinata: we now find that it prevents the hæmolytic action of this poison as well as it prevents the same action of its homologous venom. It is evident, therefore, that while the results got *in vivo* may correspond with those obtained *in vitro*, we cannot, by any means, depend on this being always the case, and that if we had been led to appraise the effect of the serum *in vivo* from the results got *in vitro*, a procedure which would appear justifiable from the testings with the venom of Crotalus adamanteus, we should have fallen into serious error. Further, it is easily calculated that the neutralising value of a specific serum for its venom as estimated by its effect in preventing the hæmolytic action of the poison *in vitro* is no criterion of its power on the general action of the poison *in vivo*. Thus, a reference to the protocols will show that 0·04 c.c. of serum was able to completely neutralise the hæmolytic action of 0·025 milligramme of daboia venom. From this it can be estimated that 1 c.c. would be able to neutralise 0·625 milligramme. Now, we have already seen that 1 c.c. of the same serum could neutralise 2·8 milligrammes of poison *in vivo*, an amount $4\frac{1}{2}$ times more than that which the same quantity of serum could neutralise when tested against the hæmolytic action.

We have now to take up the concluding portion of the present investigation, that is, the consideration of some observations which were made with the view of ascertaining if a serum prepared with daboia venom has any hindering effect on the action which some venoms exert on the coagulability of the blood plasma as tested with citrate plasma *in vitro*.

As I have pointed out previously[8] daboia venom has a marked action in increasing the coagulability of the blood, which action can also be demonstrated *in vitro* with citrate plasma. I have also shown that this action is of the nature of a ferment activity. Further, I have investigated this phenomenon with

the venoms of several other species and have found that all viperine venoms and some colubrine poisons have a similar action to that of daboia venom. On the other hand, the venoms of the cobra and of the king cobra have a direct anti-ferment action, diminishing the blood coagulability *in vivo* and delaying or completely inhibiting the clotting of citrate or oxalate plasma *in vitro*. The venoms of the following snakes with which I have worked increase the coagulability of the blood plasma, which action can be easily demonstrated with citrate plasma *in vitro* : (1) Daboia Russellii, (2) Echis carinata, (3) Trimeresurus gramineus, (4) Crotalus adamanteus and (5) Hoplocephalus curtus. If a suitable amount of any of these poisons be added to citrate plasma clotting takes place, in the case of the last four almost instantaneously. The action of daboia venom in this respect is not so marked nor so energetic as that of the others: it takes a larger quantity and a much longer time to bring about clotting than any of the other four poisons.

Daboia venom anti-serum was now tested against this action of this group of poisons. The test dose of each venom used was such an one as caused solid clotting of 2 c.c. of citrate plasma within a short time. The following was the method employed in all instances. The test dose of venom was mixed with varying amounts of serum. The mixtures were allowed to stand at laboratory temperature for half an hour. To each tube were then added 2 c.c. of citrate horse plasma (1 per cent.). The results were noted at short intervals. From table IX of the protocols it will be seen that, while the serum in small amount had a complete hindering effect on this action of its homologous venom, it had no neutralising effect on the other poisons against which it was tested. In this respect it appears to be specific. As regards the venom of Crotalus adamanteus this result appears to be in contradiction to the result obtained in the experiments made *in vivo*. It is, however, to be noted that the test dose of this poison used in the animal experiments did not produce an intravascular thumbosis : death was due to other causes.

The following conclusions may now be drawn :—

1. By injecting a horse with pure unheated daboia venom an anti-serum for this poison has been obtained. This serum is of considerable strength and has a marked neutralising effect on all the actions of daboia venom both *in vivo* and *in vitro*. It should be of considerable therapeutic value in cases of bites from this snake.

2. The serum thus obtained is markedly but not strictly specific *in vivo;* it has no action on the poisons of five colubrine snakes against which it was tested: it has a certain neutralising action on the venom of Crotalu adamanteus, but has no effect on the venoms of two other vipers, namely, Echis carinata and Trimeresurus gramineus.

3. When tested *in vitro* against the hæmolytic actions of the various poisons, it is found that this serum has no neutralising effect on this action of any colubrine venom: that it neutralises the venom of Echis carinata as well as it does that of the poison with which it is prepared: that it has a marked but not equally great effect on the venom of Crotalus adamanteus; and that it has no neutralising action on the venom of another viper, namely, Trimeresurus gramineus. Further, when tested *in vitro* against the fibrin ferment actions of various poisons, it is found that, while this serum neutralises well its homologous venom, it has no effect on the poisons of four other species, one colubrine and three viperine, namely, (1) Hoplocephalus curtus, (2) Echis carinata, (3) Trimeresurus gramineus and (4) Crotalus adamanteus.

4. These observations lead us to the same conclusion which was previously arrived at and which receives the support of Noguchi's observations,[9] namely, that an antivenomous serum is markedly but not strictly specific and that in treating any case of snake bite the homologous serum must be used.

Protocols.

The two antivenomous sera, which were used in the observations detailed below, were as follows: —

(1) The serum of a horse which had been immunised with pure unheated cobra venom: when tested *in vivo*, 1 c.c. of this serum could neutralise 0·7 milligramme of pure cobra venom.

(2) The serum of a horse which had been subjected for the period of a year to repeated doses, gradually increasing in amount, of pure unheated daboia venom.

In all the experiments to test the neutralising effect of these two sera on the general action *in vivo* of the several venoms, rabbits were used. The test dose, a small multiple of the minimum lethal dose either by subcutaneous or intravenous injection, was mixed *in vitro* with different amounts of the serum under experiment. The mixtures before injection were allowed to stand at laboratory temperature for at least half an hour.

Table I.—Experiments to ascertain if a serum prepared with pure cobra venom has any neutralising effect for the general action in vivo *of the venom of the king cobra (Naia bungarus).*

The minimum lethal dose for rabbits of the sample of venom used was 0·35 milligramme by subcutaneous injection. The test dose now used was about 6 lethal doses.

The following results were obtained :—

Animal.	Weight in grammes.	K. C. V. in milligrammes.	Serum.	Result.
Rabbit 1 . .	1,020	2	10 c.c	Died in 52 hours.
„ 2 . .	1,040	2	15 c.c.	Died in 52 hours.
„ 3 (Control) .	1,120	2	*Nil.*	Died in 2½ hours.

From this it is seen that, while large amounts of serum were able to delay death considerably, they did not completely neutralise the venom. It can be calculated that 1 c.c. of serum failed to neutralise 0·112 milligramme of poison.

TABLE II.—Experimetns to ascertain if a serum prepared with pure cobra venom has any neutralising effect for the general action in vivo *of the venom of Bungarus cæruleus.*

The minimum lethal dose for rabbits of the sample of venom used was 0·04 milligramme per kilo. by intravenous injection. The test dose now used was five lethal doses, namely, 0·2 milligramme per kilo.

The following were the results :—

Animal.	Weight in grammes.	B. C. V. in milligrammes per kilo.	Serum.	Result.
Rabbit 1 . .	1,110	0·2	5 c.c.	Died in 82 minutes.
„ 2 . .	1,180	0·2	8 c.c.	Died in 75 minutes.
„ 3 (Control) .	1,000	0·2	*Nil*	Died in 90 minutes.

From this it is seen that 8 c.c. of serum had no effect whatever even in delaying death. We can calculate that 1 c.c. of serum could not neutralise even 0·025 milligramme of venom.

TABLE III.—Experiments to ascertain if a serum prepared with pure daboia venom has any neutralising effect for the general actions in vivo *of the poisons of five colubrine species.*

(*a*) *Cobra venom.*—The test dose used was about 7 lethal doses by subcutaneous injection into rabbits.

The following was the result :—

Animal.	Weight in grammes.	C. V. in milligrammes.	Serum.	Result.
Rabbit . . .	900	2	15 c.c.	Died in 2½ hours.

(*b*) *King cobra venom.*—The test dose used was about 6 lethal doses by subcutaneous injection into rabbits. The following was the result :—

Animal.	Weight in grammes.	K. C. V. in milligrammes.	Serum.	Result.
Rabbit 1 . .	1,110	2	15 c.c.	Died in 3 hours.
„ 2 (Control) .	1,120	2	*Nil*	Died in 2½ hours.

(*c*) *Bungarus cæruleus venom.*—The test dose used was 5 lethal doses by intravenous injection into rabbits. The following was the result :—

Animal.	Weight in grammes.	B. C. V. in milligrammes per kilo.	Serum.	Result.
Rabbit 1 . . .	1,040	0·2	8 c.c.	Died in 1½ hours.
„ 2 (Control) .	1,000	0 2	*Nil.*	Died in 1½ hours.

(*d*) *Bungarus fasciatus venom.*—The test dose used was about 3 lethal doses by intravenous injection into rabbits. The following was the result :—

Animal.	Weight in grammes.	B. F. V. in milligrammes per kilo.	Serum.	Result.
Rabbit 1 . . .	1,110	2	15 c.c.	Died in 11 hours.
„ 2 (Control) .	1,290	2	*Nil.*	Died in 34 hours.

(*e*) *Enhydrina valakadien venom.*—The test dose used was 10 lethal doses by subcutaneous injection into rabbits.

The following was the result :—

Animal.	Weight in grammes.	E. V. V. in milligrammes per kilo.	Serum.	Result.
Rabbit 1 . .	870	0·5	15 c.c.	Died in 1½ hours.
„ 2 (Control) .	830	0·5	*Nil.*	Died in 1½ hours.

TABLE IV.—Experiments to determine the neutralising power of a serum prepared with pure daboia venom for the general action in vivo *of pure unheated daboia venom.*

The minimum lethal dose of the sample of venom used was 0·1 milligramme per kilo. by intravenous injection into rabbits. The test dose now used was 5 milligrammes per kilo., that is, 50 lethal doses. The following were the results. All the rabbits were about one kilo. weight.

Animal.	D. V. in milligrammes.	Serum.	Result.	Remarks.
Rabbit 1 . .	5	0·1 c.c.	Died in 2 minutes.	Intravascular clotting marked.
„ 2 . .	5	0·75 c.c.	„	Ditto.
„ 3 . .	5	1 c.c.	Died in 10½ hours.	No intravascular clotting.
„ 4 . .	5	1·25 c.c.	„ 31 „	Ditto.
„ 5 . .	5	1·5 c.c.	„ 34 „	Ditto.
„ 6 . .	5	1·75 c.c.	Recovered.	
„ 7 . .	5	2 c.c.	„	
„ 8 . .	5	3 c.c.	„	
„ 9 (Control)	5	*Nil.*	Died in 1 minute.	Intravascular clotting marked.

From this table it is seen that 1 c.c. of serum was able to prevent the intravascular clotting but was not able to save the life of the animal; also, that 1·75 c.c. was able to completely neutralise the test dose of poison. We can, therefore, calculate that 1 c.c. of serum would be able to neutralise 2·8 milligrammes of daboia poison.

TABLE V.—Experiments to ascertain if a serum prepared with pure daboia venom has any neutralising effect on the general action in vivo *of the poison of Echis carinata.*

The minimum lethal dose of the sample of venom used was 0·05 milligramme per kilo. by intravenous injection into rabbits.

Two experiments were made :—

(*a*) In the first observation the test dose used was 2 lethal doses, that is, 0·1 milligramme per kilo. The following was the result :—

Animal.	Weight in grammes.	E C. V. in milligrames per kilo.	Serum.	Result.
Rabbit 1 . . .	1,000	0·1	5 c.c.	Died in 2 minutes.
„ 2 (Control) .	1,100	0·1	*Nil.*	Died in 2 minutes.

From this it can be calculated that 1 c.c. of serum could not neutralise the general action *in vivo* of 0·012 milligramme of Echis carinata venom.

(*b*) In the second observation a single lethal dose was used as a test dose.

The following was the result :—

Animal.	Weight in grammes.	E. C. V. in milligrames per kilo.	Serum.	Result.
Rabbit . . .	910	0·03	5 c.c.	Died in 5 minutes.

TABLE VI.—Experiment to ascertain if a serum prepared with pure daboia venom has any neutralising effect on the general action in vivo *of the poison of Trimeresurus gramineus.*

There was not sufficient of this poison available to determine with accuracy the minimum lethal dose. It was, however, ascertained that in rabbits 2 milligrammes per kilo. by intravenous injection killed, while 1 milligramme per kilo. was non-lethal. The test dose now used was 2 milligrammes per kilo.

The following was the result :—

Animal.	Weight in grammes.	T. G. V. in milligrames per kilo.	Serum.	Result.
Rabbit 1 . . .	1,010	2	5 c. c.	Died in $4\frac{1}{2}$ days.
„ 2 (Control) .	1,030	2	*Nil.*	Died in $1\frac{1}{2}$ days.

It is, therefore, apparent that this serum has no neutralising effect for the general action *in vivo* of this poison.

TABLE VII.—Experiments to ascertain if a serum prepared with pure daboia venom has any neutralising effect on the general action in vivo *of the poison of Crotalus adamanteus.*

The minimum lethal dose of this venom was determined to be 0·25 milligramme per kilo. of rabbit by intravenous injection. The test dose now used was 8 lethal doses, namely, 2 milligrammes per kilo.

The following results were obtained :—

Animal.	Weight in grammes.	C. A. V. in milligrammes per kilo.	Serum.	Result.
Rabbit 1 . .	1,100	·2	1 c.c.	Died in 1½ hours.
„ 2 . .	1,200	2	2 c.c.	„ 1¾ „
„ 3 . .	1,180	2	3 c.c.	„ 1¾ „
„ 4 . .	1,000	2	4 c.c.	Recovered.
„ 5 (Control) .	1,030	2	*Nil.*	Died in 6 minutes.

From this table it is seen that 4 c.c. of serum were able to neutralise 8 lethal doses of poison. It can, also, be calculated that 1 c.c. of serum would be able to neutralise 0·45 milligramme of Crotalus adamanteus venom.

TABLE VIII.—Experiments to ascertain if a serum prepared with pure daboia venom has any neutralising effect on the hæmolytic actions of the venoms of various snakes.

The following technique was employed. Dog's blood was defibrinated : the red cells were washed several times with 0·85 per cent. salt solution. A 5 per cent. suspension of the washed red cells was made in 0·85 per cent. sterile salt solution : of this suspension 1 c.c. was the amount used in each preparation. The test dose of venom was in each instance a single complete hæmolysing dose, or a very small multiple of this amount, when a fixed quantity of serum complement was added. This test dose and varying amounts of serum were mixed and allowed to stand in contact for half an hour at room temperature. To each tube was then added 1 c.c. of the 5 per cent. red cell suspension and 0·5 c.c. of a two-fold dilution of dog's fresh serum. The preparations were left in the incubator (37°C.) for one hour and then placed in the ice chest for 18 to 20 hours, after which time he results were recorded.

The letters at the top of each column are the initial letters of the genus and species of each snake as follows :—

I.—Colubridæ.

1. Naja tripudians (cobra) = N. T.
2. Naja bungarus (king cobra) = N. B.
3. Bungarus cœruleus (krait) = B. C.
4. Bungarus fasciatus (banded krait) = B. F.
5. Hoplocephalus curtus (tiger snake) = H. C.
6. Enhydrina Valakadien (sea-snake) = E. V.

II.—Viperidæ.

7. Vipera Russellii (daboia) = V. R.
8. Echis carinata (phoorsa) = E. C.
9. Trimeresurus gramineus (green pit viper) = T. G.
10. Crotalus adamanteus (rattlesnake) = C. A.

Serum.	N. T. 0·025 Mgr.	N. B. 0·025 Mgr.	B. C. 0·025 Mgr.	B. F. 0·05 Mgr.	H. C. 0·025 Mgr.	E. V. 0·1 Mgr.	V. R. 0·025 Mgr.	E. C. 0·05 Mgr.	T. G. 0·025 Mgr.	C. A. 0·025 Mgr.
2 c.c.	+	+	+	+	+	+	...	...	+	...
1 c.c.	+	+	+	+	+	+	...	...	+	...
0·8 c.c.	+	+	+	+	+	+	...	...	+	—
0·6 c.c.	+	+	+	+	+	+	...	...	+	—
0·4 c.c.	+	+	+	+	+	+	...	...	+	—
0·2 c.c.	+	+	+	+	+	+	...	...	+	—
0·1 c.c.	+	+	+	+	+	+	—	—	+	+
0·08 c.c.	...	...	...	...	...	...	—	—	...	+
0·05 c.c.	...	...	...	...	...	...	—	—	...	+
0·04 c.c.	...	...	...	...	...	...	—	—	...	+
0·02 c.c.	...	...	...	...	...	...	+	+ —	...	+
0·01 c.c.	...	...	...	...	...	...	+	+	...	+
0·08 c.c.	...	...	...	...	...	...	+	+	...	+
Nil (Control).	+	+	+	+	+	+	+	+	+	+

\+ Complete hæmolysis : — no hæmolysis.

TABLE IX.—*Experiments to ascertain if a serum prepared with pure daboia venom can neutralise the actions on citrate plasma* in vitro *of the venoms of* (1) *Daboia Russellii,* (2) *Echis carinata,* (3) *Trimeresurus gramineus,* (4) *Crotalus adamanteus, and* (5) *Hoplocephalus Curtus.*

All these poisons clot citrate plasma *in vitro* without the addition of any

soluble salt of lime. The technique employed to test if daboia venom anti-serum had any hindering effect on this action was as follows :—

The test dose of each poison, namely, an amount which clotted the control in a short time, was mixed with varying quantities of serum. The mixtures were allowed to stand at laboratory temperature for half an hour. Then to each tube there was added 2 c.c. of citrate horse plasma (1 per cent. citrate). A control tube without serum was prepared in each instance.

The following results were obtained :—

Serum.	D. R. V. 2 Milligrs.	E. C. V. 0·05 Milligrs.	T. G. V. 0·3 Milligrs.	C. A. V. 0·05 Milligrs.	H. C. V. 0·05 Milligrs.
1·5 c.c.	Liquid after 24 hours .	Clotted 8 minutes.	Clotted 7 minutes.	Clotted 7 minutes.	Clotted 7 minutes.
1 c.c.	Ditto	Ditto	Ditto	Ditto	Ditto
0·5 c.c.	Ditto	Ditto	Ditto	Ditto	Ditto
0·25 c.c.	Ditto	Clotted 7 minutes.	Ditto	Ditto	Ditto
0·1 c.c.	Ditto	Ditto	Ditto	Ditto	Ditto
0·05 c.c.	Ditto	Ditto	Ditto	Ditto	Ditto
0·025 c.c.	Ditto	Clotted 5 minutes.	Ditto	Ditto	Ditto
0·01 c.c.	Loose clot after 24 hours	Ditto	Ditto	Ditto	Ditto
0·005 c.c.	Clotted after 24 hours .	...	...	...	...
0·0025 c.c.	Clotted after 1 hour .	...		...	...
0·001 c.c.	Clotted after 1 hour .	...	...	...	...
Nil (Control)	Clotted after 1 hour .	Clotted 5 minutes.	Clotted 7 minutes.	Clotted 7 minutes.	Clotted 7 minutes.

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